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Characterisation and antifungal susceptibility testing of Candida isolates in Mucocutaneous Candidiasis

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Abstract

Background and Objectives: The last two decades have seen a significant rise in the infections caused by drug resistant Candida species in various centers in India. The objective of this study was to isolate and speciate*Candida* species from various clinical samples, to detect their antifungal susceptibility pattern and to note down the risk factors.

Methods: *Candida* species isolated from various clinical samples were subjected to speciation using standard yeast identification protocol and CHROM agar. Antifungal susceptibility testing was done by the disc diffusion method to amphotericin B, fluconazole, nystatin, itraconazole, ketoconazole and clotrimazole. Clinical details and risk factors of the patients were noted down.

Results: Among the 100 culture positive cases,various species of *Candida* were reported as *C. albicans* (54%), *C. tropicalis* (22%), *C. glabrata*(12%), *C. krusei* (06%), *C.parapsilosis* (04%) and *C. kefyr*(02%).Resistance for *Candida* in present study was 26% for fluconazole, 24% for itraconazole, 29% for clotrimazole, 18% for ketoconazole and 10% for nystatin. No resistance was seen to amphotericin B.

Conclusion:.*Candida albicans* was the predominant isolate. Accurate identification of different species of *Candida* is essential not only for epidemiological purposes but for diagnostic and prognostic value to patients because all species do not respond to same antifungal drugs. The development of resistance to commonly used antifungal drugs indicate need for rational use, preferably after in- vitro antifungal susceptibility testing.

Keywords: Antifungal susceptibility testing, *Candida* speciation, CHROM agar, Antifungal discs

Introduction

Candida is the commonest fungal infection found in the humans affecting mucosa, skin, nails and internal organs. *Candida* species colonize the mucosal surfaces of all humans soon after birth and the risk of endogenous infection is ever-present. [4]*Candida* species are component of normal flora of human beings and commonly found on the skin, gastrointestinal tract and female genital tract particularly higher in vagina during pregnancy.[4] Carriage rate of *Candida* species tends to increase with age. *Candida* species are the fifth most common cause of blood stream infections and fourth common

cause of nosocomial infections.[4,13]Candidiasis (moniliasis) is infection with *Candida*species, most commonly *Candida albicans*. *Candida* is ubiquitous yeast that resides harmlessly on skin and mucous membranes until dampness, heat and impaired local and systemic defenses provide a fertile environment for it to grow. *Candida* species produces various cutaneous, mucocutaneous and systemic manifestations depending on immune status of host & underlying predisposing factors.

*Candida albicans*is generally considered the major pathogenic among the *Candida* species. [4,13] Increase in the prevalence of non-albicans species such as *Candida glabrata* and *Candida krusei*[23] has been noted during the past decade, because of the extensive use of anti mycotic drugs particularly azoles, for prolonged periods. *Candida glabrata* is associated with severe complications than other species.[23] Epidemiological surveys reveal that *C.tropicalis*and *C.glabrata*are the second most frequent species isolated; the former from the oropharynx and the later from the vagina and the GI tract.

The incidence of fungal infections is increasing at an alarming rate. This increase is directly related to the growing population of immuno compromised individuals, resulting from changes in medical practice such as the use of intensive chemotherapy, radiotherapy and immunosuppressive drugs. HIV and other diseases which cause immunosuppression have also contributed to this problem. Oropharyngeal candidiasis (OPC) occurs primarily in individuals with HIV. Clinically OPC is most common when CD4 + T cell count drops below 200 cells/cu mm.[4]

Early speciation of positive clinical specimens has immense potential to impact the therapeutic decisions regarding empirical antifungal therapy. Systemic infections due to yeasts and resistance to antifungals are on the rise in Indian hospitals. [5] Increasing resistance to azoles and amphotericin B has been reported both from India and other countries. [5,16] *C. krusei* and *C. glabrata* are known for their innate resistance to fluconazole.[10] Therefore, species identification with antifungal susceptibility pattern of Candida isolates helps in the selection of appropriate antifungal agents, successful treatment, in antifungal prophylaxis in the immuno compromised host and to prevent the emergence of drug resistance.[8,14] So, in this study, our aim was to determine the species level distribution and antifungal susceptibilities of *Candida* strains from various mucocutaneous clinical samples. This would be of immense benefit to the patient as well as an important & valuable tool.

Materials and methods

The study was conducted at the Department of Microbiology, Government Medical College and M.B.S hospital, Kota from June 2012 to Sept 2013. A total of 100 strains of *Candida* were isolated from various clinical samples of patients admitted in MBS Hospital & associated Group of Hospitals - JK Lone Hospital & NMC Hospital, Kota, Rajasthan.

Samples were collected from both male and female patients of different age groups. Different clinical samples included oral thrush, vaginitis, balanitis and angular cheilitis.*Candida* species isolated from various clinical samples were subjected to speciation and antifungal susceptibility testing. The risk factors of the patients were noted down. The Standard Protocol for yeast identification included the following tests : Gram's stain, KOH mount, India ink preparation, Culture on Sabouraud's Dextrose Agar, GermTube test, Cornmeal agar morphology (Dalmau technique), Sugar fermentation test, Sugar assimilation test (Auxanographic Plate Method)[18,27] and CHROM Agar morphology[9,20]. Antifungal Susceptibility Test was done using the National Committee for Clinical Laboratory

Standards, 2004 method for antifungal disc diffusion susceptibility for yeasts with approved guideline M44-A2.[17] We used Mueller Hinton Agar (MHA) supplemented with 2% glucose and 0.5 µg/ ml methylene blue dye and following antifungal discs: fluconazole (25µg), nystatin (100 units), amphotericin B (100 units), ketoconazole (10µg), itraconazole (10µg) and clotrimazole (10 µg). The inoculum was standardized to 0.5 Mc Farland units.The antifungal discs were applied with the help of sterile forceps. The plates were incubated at 30^{o} C for 24hrs. Zone diameters were read at a point where the growth decreased.All dehydrated culture media and antifungal discs were procured from Hi-Media, Mumbai

After the measurement of zone of inhibition, the results of antifungal sensitivity were interpreted according to criteria given with HiMedia antifungal discs.

Results

In this study, total 100 isolates of *Candida*species from various clinical specimens were speciated and their antifungal susceptibility was found. The prevalence of *Candida albicans* and non albicans*Candida* were studied.The samples collected were swabs from oral thrush, vaginitis, balanitis and angular cheilitis. The results of this study have been presented in Tables I - IV

Antifungal Drugs	***C. albicans* (mm)**	***C. parapsilosis* (mm)**	***C. tropicalis* (mm)**	***C. krusei* (mm)**	***C. glabrata* (mm)**
Itraconazole (10µg)	16-20	11-18	8-13	8-15	12-16
Nystatin (100U/disc)	19-27	16-25	16-21	15-20	15-27
Ketoconazole (10 µg)	20-32	14-29	17-28	10-14	17-30
Fluconazole (25 µg)	27-38	22-33	16-25	9-35	17-30
Amphotericin B (100 U)	10-17	11-20	8-12	9-12	13-18
Clotrimazole(10 µg)	18-32	16-32	10-20	14-24	14-17

Table I: Details of clinical samples collected from different age group

Clinical condition	**Age groups in years**				**Total No.**
	0 – 20	**21 – 40**	**41 – 60**	**61 – 80**	
Oral thrush	05 (9.80%)	16 (31.38%)	20 (39.21%)	10 (19.61%)	54
Vaginitis	00	30 (78.94%)	08 (21.05%)	08 (21.05%)	38
Balanitis	02 (40%)	02 (40%)	01 (20%)	01 (20%)	05
Angular cheilitis	01 (33.33%)	00	02 (66.66%)	02 (66.66%)	03
Total	08 (08%)	48 (48%)	34 (34%)	10 (10%)	100

Table II: Details of Clinical Samples

Clinical condition	**Total no. (100)**
Oral thrush	54
Vaginitis	38
Balanitis	05
Angular cheilitis	03

Table III: Candida species isolated from different clinical conditions

Clinical condition	*C. albicans*	*C. tropicalis*	*C. glabrata*	*C. krusei*	*C. parapsilosis*	*C. kefyr*	Total
Oral thrush	23 (42.5%)	17 (31.4%)	06 (11.1%)	06 (11.1%)	00	02 (3.7%)	54 (54%)
Vaginitis	26 (68.4%)	03 (7.8%)	06 (15.7%)	00	03 (7.8%)	00	38 (38%)
Balanitis	04 (80%)	00	00	00	01 (20%)	00	05 (05%)
Angular Cheilitis	01 (33.3%)	02 (66.7%)	00	00	00	00	03 (03%)
Total	54 (54%)	22 (22%)	12 (12%)	06 (06%)	04 (04%)	02 (02%)	100

Discussion

Candidiasis is an opportunistic fungal infection, pathogenic species of this genus are found as commensals on skin and mucous membrane of human beings. Candidiasis has emerged as an alarming opportunistic disease as there is an increase in number of patients who are immunocompromised, aged, receiving prolonged antibacterial and aggressive cancer chemotherapy or undergoing invasive surgical procedures and organ transplantation.[15] *C. albicans* is the most common pathogenic species of *Candida* infecting man.[21,24,26]

The demographic data in present study showed that the maximum number of *Candida* positive samples were from the age group 21-40 years (Table I).This data was primarily due to high incidence of *Candida* isolated from female patients of child bearing age suffering from vulvovaginitis. It was in consonance with the findings of Sunil Kumar Birader et al[2] who stated that the high hormone level leads to a proportional increase in the glycogen content of the vagina that favour colonization by *Candida* in pregnancy. Table II shows various clinical conditions of mucocutaneous Candidiasis from which *Candida* species were isolated. Among the 100 *Candida* isolates, maximum number were isolated from oral thrush cases (54%) followed by vaginitis (38%), balanitis (05%) and angular cheilitis (03%). The major predisposing factors for oral thrush in present study were malnutrition and debilitated state of patients due to malignancy, prolonged stay in ICU, secondary to chronic infections, diabetes and prematurity. Leukemia patients undergoing chemotherapy present an optimal environment for the development of oral candidiasis, especially during periods of neutropenia. *Candida* species are responsible for approximately one-half of all oral infections occurring during antileukemia chemotherapy.[6]

High *Candida* isolation in vaginitis was primarily due to pregnancy, sexually transmitted infections and secondary to other debilitating conditions. This high incidence of oral thrush and vaginitis correlated with study done by Sunil Kumar Birader et al, (2009).[2]

Table IV: Antifungal Sensitivity Profile of *Candida* Isolates (in percentage)

Anti fungal drugs	Fluconazole (25 µg)			Itraconazole (10 µg)			Clotrimazole (10 µg)			Ketoconazole (10 µg)			Nystatin 100 U/disc			AmB 100U		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
C. albicans	48.1	29.6	22.2	29.6	44.4	25.9	14.8	55.5	29.6	29.6	44.4	25.9	66.7	29.6	3.7	100	00	00
C. tropicalis	54.5	31.8	13.6	54.5	31.8	13.6	40.9	36.3	22.7	90.9	9.1	00	40.9	36.3	22.7	100	00	00
C. glabrata	50	16.6	33.4	50	16.6	33.4	16.6	33.4	50	50	33.4	16.6	33.4	50	16.6	100	00	00
C. kefyr	100	00	00	100	00	00	100	00	00	100	00	00	100	00	00	100	00	00
C. parapsilosis	50	25	25	50	25	25	25	50	25	50	25	25	25	50	25	100	00	00
C. krusei	00	00	100	00	00	100	66.6	16.6	16.6	66.6	16.6	16.6	100	00	00	100	00	00
Total (in percentage)	48	26	26	38	38	24	26	45	29	50	32	18	58	32	10	100	00	00

S – Sensitive, I – Intermediate sensitive, R – Resistant

Table V: Comparison of various studies showing *Candida* species recovered from clinical samples (in percentage)

	Present study	**Ompra kash Bobade et al 2013[3]**	**Saldhei Z et al 2012[22]**	**Vijaya D et al 2011[26]**	**Ragini Ananth Kashid et al 2011[11]**	**P K Felgo et al 2011[7]**	**Deepa Babin et al 2011[1]**	**Shiva nand et al 2011[21]**	**Fouzi a et al 2010[12]**	**Sunil k Birade r et al 2009[2]**	**Usharan i et al 2005[25]**
C. albicans	**54**	36.6	79.1	45.9	29.2	49.3	35.5	47	30	61.3	62.5
C. tropicalis	**22**	22.9	5.9	35.29	46.2	11.9	22.9	30	21	18	15.6
C. glabrata	**12**	13.7	11.9	-	6.12	17.9	20.6	09	08	10.6	9.3
C. krusei	**06**	08.7	2.9	10.78	-	4.5	15.7	14	03	3.3	-
C. parapsilosis	**04**	03.6	-	7.84	10.2	1.5	-	-	10	-	-
C. kefyr	**02**	11.46	-	-	1.36	-	-	-	-	-	-

C. albicans (54%) was the most prevalent species of *Candida* reported in present study (Table V). This finding was consistent with the findings of other workers who reported that the incidence of *C. albicans* was 61.3% (Sunil K Birader et al)[2], 49.3% (P K Felgo et al)[7] and 47% (Shivanand et al).[21] However, RaginiAnanthKashid et al[11] found that *C. tropicalis* was the most prevalent species accounted for 46.2% followed by *C. albicans* 29.2%.*C. tropicalis* (22 %) was the second most common species reported in present study. This finding was comparable with other workers, Bobade O et al[3] (22.9%), Babin D et al[1] (26.4%), Fouzia et al[12] (21%). However, *C. glabrata* was reported as second most common species by Felgo P K et al[7](17.9%)and Saldhei et al[22](11.9%).

The most common antifungal drugs in the current clinical use, for treatment of oral candidiasis are polyenes (amphotericin B and nystatin) and azoles (miconazole, fluconazole, ketoconazole, and itraconazole), which are mainly used topically.[19] The therapeutic and prophylactic use of antifungal agents has given rise to alarming cases of antifungal resistance. Overall antifungal drug resistance for *Candida* in present study was 26% for fluconazole, 24% for ftraconazole, 29% for clotrimazole, 18% for ketoconazole and 10% for nystatin. No resistance was observed for amphotericin B. In present study 22.2% of *C. albicans* were found to be fluconazole resistant which is in consonance with Kashid R A et al[11] and Babin D et al.[1] However, a higher resistance was observed by Saldhei et al[22] (81.1%). For NAC the resistance varies from 0 % in *C. kefyr* to 100% in *C. krusei*. For AmB no resistance was observed in present study. This was in consonance with Kashid R A et al.[11]Bobade O et al[3] reported 7.5% resistance in *C. albicans*.

Accurate identification of different species of *Candida* is essential not only for epidemiological purposes but for diagnostic and prognostic value to patients because all species do not respond to same antifungal drugs. The development of resistance to commonly used antifungal drugs indicate need for rational use, preferably after in-vitro antifungal susceptibility testing.

Conclusion

The species level identification of the *Candida* isolates along with their antifungal susceptibility patterns can greatly influence the treatment options for the clinician and may have an impact on the patient care. Antifungal susceptibility testing must be a mandatory part of the laboratory workup of every *Candida* isolates.

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